or *suspension* (because many particles – intact organelles or fragments of organelles – are suspended in the solution). In the nonaqueous approach, water was removed from the material by means of freeze-drying, and then milling and grinding were used to break the cell membranes. Although the nonaqueous method was the first to be employed (by Bensley and Hoerr, for example), and manifested considerable advantages, especially for studying the nucleus, its disadvantages made it less suitable for studying cytoplasmic structures. Perhaps most critical was that the drying process utilized chemical agents that could inactivate many of the enzymes researchers were targeting. Since, following Claude, the aqueous techniques came to be preferred in studies of cytoplasmic organelles, I will focus on them and especially on two key elements: the means of breaking cell membranes and the media in which the contents were maintained.

### *Breaking Cell Membranes*

Membranes – the cell membrane (plasma membrane) as well as various internal membranes – are relatively tough and difficult to break. Moreover, it is important to break the cell membrane without destroying the membrane's surrounding internal organelles, because breaking these could cause a redistribution of the enzymes residing in the organelles. Although its existence was not even anticipated as the earliest work on cell fractionation was being pursued, the lysosome provides a particularly vivid example of the hazards of breaking internal membranes. The lysosome contains hydrolytic enzymes, such as acid phosphatase, which are destructive of other cell organelles. Breaching the lysosome membrane would result in the rapid destruction of the other cell organelles under investigation. The desire not to destroy internal membranes largely ruled out use of the Waring blender, which was a standard tool for preparing homogenates in biochemistry. (In the dominant paradigm in biochemistry, the cell was regarded as essentially a sac of enzymes. To bring enzymes into solution for study, it was desirable to break any membranes that kept them isolated within organelles.) In addition to the objective of breaking all and only cell membranes, a further concern was the speed of operation; any delay between the initial breaking of cells and centrifugation could result in changes in the cell contents. So the goal was relatively straightforward – quickly break all cell membranes but not the membranes of any internal organelles.

Early researchers explored several different means of breaking the cell membrane. Emphasizing gentleness, in his early studies Claude gently rubbed